Rough Draft

words sheets for revision of new rough draft

New research to Doctor for review

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INTRODUCTION

White pine blister rust (Cronartium ribicola, Fischer) is controlled by eradicating *ribes from the area within a white pine (Pinus monticola, Douglas) stand and a 300 to 900 foot wide strip, adjacent.

It is most difficult and near impossible to find and kill all ribes present on a control area the first time the area is worked.

To determine the effectiveness of ribes eradication in controlling white pine blister rust it is necessary to identify the cankers of pine infection and determine the year of inception accurately. Cankers originating after control work are directly chargeable to the remaining ribes.

There is an incubation period in the life cycle of the rust on pine which will be referred to and called, in this paper, the incubation period. This incubation period is defined as that time from infection of the needle to appearance of discoloration of the bark at the base of the infected needle. This is in accord with Lochmund (1933) and Mielke (1943).

The incubation period has never been accurately determined in western white pine of the different age classes. In fact, the rust incubation period has not been conclusively established for any species of white pine.

The purpose of this study is to further investigate the length of this period in young western white pine.

*The common name "ribes" and the generic name "ribes" are used herein to indicate both currants and gooseberries.

ORIGIN OF THE BLISTER RUST FUNGUS

Dietrich found the first known specimens of the blister rust fungus on ribes and pine in the Baltic Provinces of Russia in 1854.

The blister rust fungus is probably native to North Asia (Spaulding 1922, 1929; Moir 1924; Tubeuf 1936; Mielke 1943). It became well-established in Northwestern Europe on white pine woodlots and nurseries by 1900.

INTRODUCTION TO NORTH AMERICA

The fungus was shipped on white pine planting stock from Europe into Northeastern North America (Spaulding 1922, 1929; Mielke 1943) about 1902 and definitely into Northwestern North America in 1910. It is now well-established in the white pine forests of the United States with the probable exception of those located in Utah, Nevada, Colorado, Arizona and New Mexico.

THE DISEASE

The blister rust disease of Western white pine (Pinus monticola, Douglas) is caused by the parasitic fungus Cronartium ribicola, Fischer. The fungus completes its life cycle on two different kinds of host plants, ribes and 5-needle pines. It becomes established on ribes by aecial spores which are produced in the bark of 5-needle pines and carried by air movement to the ribes leaves. It invades the five-needle pine by basidiospores that are produced on the under side of ribes leaves and carried to the pine needles by air movement.

There are certain factors that contribute to the spread and development of blister rust. The more important of these factors (Hirt 1935, 1942; Mielke 1943), are weather conditions favorable for aecial sporulation in early spring, air current movement favorable to the dissemination of aeciospres at time of heavy production, moisture and temperature conditions favorable for the germination of

aeciospores and subsequent infection of ribes, conditions favorable for maximum production of sporidia, and weather conditions favorable for pine infection. These conditions are all present at the proper time in the life cycle of the rust in seasons of pronounced infection.

HOST PLANTS

Information accumulated to date shows that all white pine species native to North America are susceptible to the blister rust fungus. Investigators (Hirt 1940; Mielke 1943; Childs and Bedwell 1947) found considerable differences in the degree of susceptibility between some of the white pine species.

Most of the ribes occurring in the white pine forests of North America have been tested for blister rust tentative susceptibility and teliospore production (Hahn 1928; Mielke and Hansbrough 1933; Mielke, Childs and Lochmund 1937; Kimmey 1938; Mielke 1943) under natural conditions or controlled inoculations. Based on this evidence, none of these ribes species were found to be immune to the blister rust fungus, but wide differences in susceptibility and teliospore production were observed.

PREVIOUS WORK

The fungus infects white pine through the needles and ribes through the leaves by air-borne basidiospores and aeciaspores respectively (McCubin 1917; Colley 1917, Clinton and McCormick 1919; Spaulding 1929; Lochmund 1933; Pierson and Buchanan 1938; Hirt 1938, 1939, 1942).

After the fungus enters the white pine needle (Clinton and McCormick 1919; Chapman 1934; Hirt 1942) a compact mass of hyphae develops below the point of entrance before hyphal growth advances through the vascular bundle of the needle in a proximal course into the bark. The first appearance of the blister rust

fungus on the stem by the unaided eye is a yellowish to orange colored ~~spot~~ in the bark around the base of the needle a foscicle. The natural greyish-green bark fades to the yellowish or orange color after being parasitized by the mycelium which spreads (Lochmund 1933; Erlich and Oppie 1940; Hirt 1942) in a girdling and proximal manner, usually (Lochmund 1933) **damaging or killing the tree.

THE PROBLEM

The inconspicuous period from infection of the needle by the sporidial germ tube to the appearance of yellowish to orange colored bark at needle base is the period of time in the life cycle of the rust on pine that is in question and it is this period that we wish to investigate.

The study of the movement of the mycelium of white pine blister rust (C. ribocola, Fischer) in young western white pine (P. monticola, Douglas) is conducted for the purpose of securing basic information during the incubation period.

Knowing the rust incubation period, it will be possible to determine the year and season rust originated and thus know if infection occurred before or after ribes eradication.

From these determinations the effectiveness of ribes reduction in controlling the rust may be measured.

MATERIALS AND METHODS

An area near Bovill, Idaho was carefully examined and found apparently free of ribes and supporting a thrifty stand of young western white pine reproduction.

On this area 46 healthy native western white pine 5 to 7 years old and 7 to 15 inches high were selected for observation.

**Killing or damage sufficient to render trees worthless for timber or timber production.

September 7, 1933 these 46 pines were carefully examined for the presence of the blister rust fungus and none was in evidence to the unaided eye. The 46 pine were immediately dug and carefully wrapped and ~~under cover~~ transported several miles to Deep Creek in the vicinity of Elk River, Idaho. Here they were potted with native soil in 8 inch earthen pots. Twelve of the potted pine were immediately returned to the area from where they were dug and used as controls.

The remaining 34 pine were moved to the edge of the stream course within 6 feet of the water in Deep Creek, the same day they were dug, potted and transported.

SOURCE OF INOCULUM

An abundance of native Ribes petiolare infected with the blister rust fungus in the telium and uredial stage were within ~~a few inches~~ two feet of the potted pines, ~~the ribes forming a canopy above.~~ lower

LENGTH OF EXPOSURE AND PREVAILING WEATHER

The 34 pine were exposed as described in the above section from September 7, 1933 to October 11, 1933. Rainfall occurred intermittently over this area between September 9 and 24 inclusive.

The temperature range and moisture condition were favorable (Hirt 1935, 1942) for sporidial production and pine infection throughout the 16 day period. However,, because of teliospore exaustion (Hirt 1942) it is unlikely that little if any pine infection took place after September 11 because of telial and sporidial depletion. Although the 16 day period was favorable for sporidial cast it was not favorable for the production of a new crop of teliospores. ~~After September 24 weather was not favorable for the production of a new crop of teliospores,~~ (see this sentence) After September 24 weather was ~~not~~ it favorable for pine infection.

DISPOSITION AND LOCATION

On October 11, the 34 exposed pine and the 12 check pine were transported to the University of Idaho at Moscow for observation.

They were kept in a windbreak with north exposure during the winter months which was somewhat comparable to native forest conditions occurring in their original location. In June 1934 they were transplanted on a plot near Newman Lake Washington for further inspection.

EXAMINATION OF PINE

Careful observations were made daily from October 11 to November 9 to detect the first evidence of needle infection. Thereafter periodical examinations were continued through 1934 and 1935. No additional evidence was found in 1934 to explain that no new cankers developed after Oct. 12 1934 and no new cankers were found in 1935. The first evidence of infection was definetely identified and verified microscopically on November 9, 1933 by E.E. Hubert and R.K. Pierson.

The first appearance of these minute infection spots on the needles appear almost transparent-lemon color against the needle-green background but within 2 or 3 weeks after appearance they acquire a translucent lemon-yellow color and 6 to 8 months later, take on a golden-yellow hue which gradually turns brown before needle cast. When these spots first appear they are tiny specks showing only on one side of the neeld but progressively enlarge. If the needle is not cast within a year they usually occupy a complete section of the needle often a M.M. in length..

COLLECTION OF NEEDLES

All exposed pines developed blister rust infection in varying amounts but the 12 controls remain free of blister rust infection.

November 9, 1933 to April 30, 1934, 10 to 25 needles with blister rust infection spots were collected approximately every 10 days and fixed in formal-acetic alcohol.

All needle age classes present were collected in a rotating manner over the 34 pines.

LABORATORY TECHNIQUE

collected needles were fixed in formal - acetic acid - alcohol.

Fixed needles were prepared in three M.M. length, dehydrated with alcohol, cleared with xylene, infiltrated with soft parafin, imbedded in hard parafin, sectioned with a rotary microtome. The sections were fixed on slides with Haupt's fixative, stained with saffronine-red and fast-green, mounted on slides with Canadian balsin and examined with 100X to 2500X range miscroscope for the presence of the fungus.

SIGNIFICENT DATES

September 9, 1933—First date favorable for blister rust infection.

September 11, 1933—Last date blister rust infection likely took place.

November 9, 1933—First appearance of blister infection spots on needles.

January 24, 1934—First proximal movement of blister rust mycelium.

March 5, 1934——Blister rust mycelium reaches needle base.

April 2, 1934——First appearance of orange colored bark at needle base.

June 27, 1934——First appearance of pycniospores.

October 12, 1934—Last cankers to appear.

April 1, 1935——First aeciospores produced.

No additional cankers were found in 1935 so it is assumed that no new cankers developed after Oct. 12, 1934

see page 7

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Spokane, Washington
May 20, 1947
CMC/hh

should this sheet be near the summary?

** Periods in the development of the fungus

RESULTS

1. First needle spots appeared *2.0 months from time of initial infection.

2. First proximal movement of mycelium was observed 4.5 months after initial infection.

3. From first proximal movement of mycelium until invasion of needle sheath, 1.3 month.

4. From first invasion of needle sheath by mycelium until appearance of orange colored bark at base of needle, 1.0 month.

5. From initial infection until appearance of incipient canker at needle base, 6.8 months.

6. From the appearance of first canker until the appearance of last canker, 6.4 months.

7. From initial infection to first pycnia, 9.7 months.

8. From initial infection to the appearance of last canker 13.2 months.

9. From initial infection to the appearance of Aecia, 19.0 months.

10 all pine were dead by September 1936

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*A month will be figured on the basis of 30 days ~~and referred to in this paper on that basis.~~ and the date of pine inception will be figured as of oct 11, 1933 and referred to in this paper on that basis

ACTIVITY OF THE FUNGUS IN THE NEEDLE

Microscopic examination of the fungus in the needle reveals that the incubation period occurs in four distinct phases of activity in the needle. They are the entrance of the sporidial germ tube to within the needle, the development of a mass of mycelium between the mesophyll ~~calls~~ cells and the endodermal cells, the extension of the mycelium into and in a proximal direction along the vascular system to the needle base and the continuation of the mycelium development through the needle base and into the cambium layer. In approximately four weeks after bark invasion at the needle base, the ~~bark-green~~ greyish-green of the bark color gives way to the ~~orange~~ yellowish-orange color that is the first visionable evidence of the fungus' presence in the bark by the unaided eye.

**

RELATION OF INFECTION TO AGES OF NEEDLES AND LOCATION OF INFECTION ON NEEDLE

A total of 315 infected needles of all age classes present were collected in a rotating manner over the 34 pines ~~on 19 different dates~~ from Nov 9 1933 to May 10 1934 approximately every ten days. On these needles there were 473 infections or needle spots. The distribution of the needles and infections according to the age of needles at time of exposure and the location of the infections on the needle are shown in Table No. 1. The location of the infection on the needle determines to some extent the length of incubation period.

many dates ?

Insert Table No. 1

Of the 315 infected needles that were collected 230 were prepared and microscopically examined for the fungus presence within the needle. Under each of these spots there was observed a definite mass of hyphae (Clinton and McCormick 1919) between the mesophyll cells and the endodermal sheath. Apparently it is

the development of this hyphal growth that causes needle tissue to change color near the point of infection. Also, it is evident that the enlargement of the hyphal mass, with its accompanying storage of reserve food and the ultimate rupturing of the layer of endodermal cells, is essential for the further progress of the fungus in the needle. After the expanded mass of hyphae creates a passage-way between these cells for the extension of mycelial strands, there is apparantly little or no remaining resistance to the progress of the strands down the vascular system to the needle base. Progress of the mycelium in the examined needles is indicated by the data in Table No. 2.

Insert Table No. 2

Since it is calculated that infection took place between September 9, and 11, 1933; time after pine infection will be reckoned as of and after September 12, 1933.

The first needle spots appeared 2.0 months after the time of initial infection. By January 24, 1934, 4.5 months after initial infection, the proximal movement of mycelium strands through the phloem side of the vascular system had begun. March 5, mycelium strands reached the needle base. This is 5.8 months after initial infection and 1.3 months from first proximal movement to the invasion of needle sheath. Time from first invasion of the needle sheath until the first appearance of the ~~orange~~ yellowish-orange colored bark at needle base was 1.0 month, and from initial infection until appearance of insipient canker at needle base, 6.8 months. From the appearance of the first canker until the appearance of the last canker 13.2 months. Periodical inspections through 1935 showed that no new cankers developed after October 12, 1934.

First pycnia appeared 9.7 months after initial infection and aeiospores were produced 19.0 months after initial infection.

DISTRIBUTION OF CANKERS BY YEAR OF GROWTH INFECTED

The distribution of cankers by the year growth reveals that internodes of all ages that bore needles during exposure and retained them 13.0 months ultimately supported cankers. The highest percentage of cankers developed on one year old growth, current growth bore next to the highest percentage and was followed, in order, by two and three year old growths. This is in close agreement with Lochmund (1933); Hirt (1938) and Pierson and Buchanan (1938). It appears that there is a difference in the relative susceptibility to the fungus of the different aged needles as shown in Table No. 3.

Insert Table No. 3

MYCELIUM RATE OF GROWTH AND PROGRESS

From the data on extent of mycelium growth and development in sectioned needles, it is computed that after hyphal mass breaks through the endodermal cells of the needles its average rate of proximal growth in the vascular system is approximately 6.8 M.M. per month. Since the average needle length was computed as 51.2 M.M., an average total of 7.5 months is required for mycelium strands to travel from needle tip to sheath.

Adding one month for the development of the fungus in the needle base and cambium layer prior to the appearance of insipient canker, the average total time from needle tip to insipient canker is 8.5 months, allowing 8.5 months from January 24, the date mycelium strands were noted in vascular system, it is mathematically probable that most of the cankers should have been visible in 13.0 months or by October 12, 1934.

CHECK STUDY

To supplement the data secured from the pines exposed in 1933, a second lot

consisting of 32 pines of similar age and size, from the same area, and exposed to R. petiolare infected with the blister rust fungus in the telium and uredial stage from August 10 to September 10, 1934.

The first and only weather favorable for pine infection during exposure occured September 9 and 10. It is calculated that infection took place during this period and time after pine infection will be reckoned as of and after September 11, 1934.

The first insipient cankers became visible May 21, 1935, 8.4 months after initial infection. No insipient cankers were found in 1936 during inspection season, April through October, 1936.

DIFFERENCE IN INCUBATION PERIOD

The incubation period of the last lot of pines was 1.6 longer than the first lot of pines.

It is believed that the 1.6 months difference in length of incubation period for the two groups was due mainly to differences in weather during the two winters. During the winter months of 1933-34, the weather was extremely mild with temperatures above zero on most days. During the winter months of 1934-35, sub-zero temmperatures and heavier snowfall prevailed. It is believed that the extremely mild weather conditions during the winter of 1933-34 were conducive to a more rapid development of the fungus.

There is enough evidence in this study with the two lots of pines to indicate that weather influences the development of the fungus in the pine needle to some extent.

The results of this study cannot be applied directly to studies with older trees. However, there is in these results some indication that at least a small percentage of the cankers, incubated in the same age needles, on older trees should be visible at the end of the first 13.0 months after initial infection. There is evidence which has been verified by the writers on the "Damage to Pine"

study plot at Cheekeye, British Columbia, that the incubation period for rust in pine under 10 years of age is shorter than in older stock. This is in accord with Patnoma (1932) Lachmund (1933B), Hirt (1939) and Mielke (1943).

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Rough Draft
Spokane, Washington
May 23, 1947
CMC/hh

Division of
Plant Disease Control
Blister Rust Control

618 Realty Building
Spokane 8, Washington
May 22, 1947

Mr. Edward L. Joy
Room 206 Federal Building
Cambridge 39, Mass.

Dear Ed.:

I have time to work on the manuscript, "A Study of the Incubation of the White Pine Blister Rust Fungus in Young Western White Pine" that you submitted to Dr. Martin for approval.

You will note many changes in the manuscript, as suggested by Dr. Martin.

Pierson and Buchanan published a paper in Phytopathology dealing with material mentioned in the manuscript.

It is well that dates and statement in the manuscript conform with the published paper.

Many changes in the revised manuscript should be made by you before passing it on for review.

I don't believe it necessary to use pictures of any kind and it may be possible to cut out a table or two.

I'll mail the manuscript under separate cover soon.

The field season is on here and my time will be pretty well taken up in collecting data.

Sincerely,

Clarence M. Chapman
Pathologist, Plant Disease Control

CMC/t